NOTES ON REGENERATION IN TUBULARIA CROCEA.

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The following experiments on *Tubularia* (*Parypha*) *crocea* were made during the summer of 1903, at Woods Holl, Mass., while I was occupying a research room of the Carnegie Institution in the Marine Biological Laboratory. The work was done under the direction of Prof. T. H. Morgan to whom I am indebted for many helpful suggestions.

I. The Effect of the Earlier Closing of One End of a Long Piece of the Stem of Tubularia.

In experimenting on the European hydroid *Tubularia mesembryanthemum*, Morgan (10) allowed the ends of long pieces of the stem to close and then, after an interval of from one to eight hours, he cut the pieces transversely through the middle region so that two new cut surfaces were exposed (Fig. 1, *B*, *C*). As a result, the aboral development of the proximal piece *CD*, was hastened. In many cases a polyp appeared on the aboral surface, *D*, as soon as did a polyp on the oral end, *C*, and in a few pieces a hydranth developed at *D* as early as did the hydranth on the distal end of the anterior piece, *AB*. This result is explained by Morgan as follows: "When a piece is cut in two in the middle one, two, three or more hours after its ends have closed, the influence of the oral end is temporarily removed, and the aboral end, which now has a start on the new oral end, may gain the ascendency and be the first to produce a polyp. Often, however, the polarity of the piece is sufficiently strong to give the precedence to the influences acting on the oral end. When the two influences are equally balanced, two hydranths may simultaneously develop."

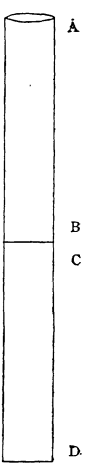

FIG. 1.

In repeating these experiments on the American hydroid,

Tubularia crocea, the hydranths already present at the anterior ends of the stems were removed by a transverse cut about 2 mm. behind the proximal circle of tentacles, and then pieces of stem varying in length from 30–40 mm. were cut off and the ends allowed to close. After an interval of from two to eight hours the stems were cut through the middle as in Fig. 1, *B*, *C*, thus producing a freshly cut surface (Fig. 1, *B*) at the aboral end of a distal piece, and also one at the oral end (Fig. 1, *C*) of a proximal piece. The results of this series of experiments are given in Table I. to IV. The first column gives the total number of individual stems operated upon; the second column shows the time that elapsed between the removal of the hydranth and the cutting of the stem through the middle; and in the following columns the results two, three, and four days after the operation are indicated. *Hy.* signifies the regeneration of a complete hydranth; *t. a.* indicates the formation of tentacle anlagen only; while *O* is used to indicate that no regeneration had taken place when the observations were made. The letters *A*, *B*, *C* and *D* refer to surfaces thus marked in Fig. 1; and the numbers in parentheses show the number of cases in which similar results were obtained.

TABLE I.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
6	2 hours.	*A* ...*hy.* (6) *B* ... *O* (6) *C*.... *t. a.* (3), *O* (3) *D*... *O* (6)	*A* *B* .....same. *C*.... *hy.* (4), *t. a.* (2) *D*... *t. a.* (2), *O* (4)	*A* *B* .....same. *C*.....*hy.* (6) *D*... *hy.* (2), *t. a.* (1), *O* (3)

Owing, probably, to differences in the temperature of the water in which they live, regeneration in *Tubularia crocea* is much slower than in *Tubularia mesembryanthemum*. In the former species a new hydranth rarely develops until two days after the removal of the old hydranth, while in the latter species a new hydranth frequently regenerates in the course of twenty-four hours. In all of the experiments in this series, as shown in the above table, a polyp formed on the oral end, *A*, of the anterior

piece, *AB*, before one developed on the oral end, *C*, of the proximal piece, *CD*. This result might, of course, be expected as the oral end, *A*, closed two hours before the end, *C*. In no case did a polyp develop at the aboral end, *D*, of the proximal piece as soon as one formed at the oral end, *C*. A start of only two hours, however, is sufficient, in most cases, to cause the formation of a hydranth at *D* before one develops at the aboral end, *B*, of the distal piece, *AB*.

The results obtained when the intervals between the cuttings were four, six and eight hours are given in Tables II. to IV.

TABLE II.

Number of Individuals.	Interval between Cuttings	Result in Two Days	Result in Three Days.	Result in Four Days.
10	4 hours.	A ... hy. (6), O (4); B ... O (10); C ... t.a. (1), O (9); D ... O (10)	A ... hy. (6), t.a. (4); B ... O (10); C ... hy. (2), t.a. (5), O (3); D ... t.a. (1), O (9)	A ... hy. (10); B ... t.a. (2), O (8); C ... hy. (7), t.a. (3); D ... hy. (1), O (9)

TABLE III.

Number of Individuals.	Interval between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
8	6 hours.	A ... hy. (3), t.a. (3), O (2); B ... O (8); C ... hy. (1), O (7); D ... O (8)	A ... hy. (6), t.a. (2); B ... O (8); C ... hy. (5), t.a. (3); D ... t.a. (4), O (4)	A ... hy. (8); B ... O (8); C .... hy. (8); D ... hy. (4), O (4)

TABLE IV.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
12	8 hours.	A ... hy. (6), t. a. (4), O (3); B ... O (12); C .... O (12); D ... O (12)	A ...hy. (12); B ...O (12); C ... hy. (10), t. a. (2); D ... hy. (2), t. a. (3), O (7)	A ...hy. (12); B ...O (12); C....hy (12); D ... hy. (5), O (7)

There is a great similarity in the results of these experiments. In all cases a hydranth formed on the oral end, *A*, of the anterior piece before one developed at any other cut surface, and the rate of development of a polyp from the aboral surface, *D*, was more rapid than that from the aboral surface, *B*, even in those cases in which *D* had only two hours start over *B*. In no case, however, did a polyp form at *D* before one developed at *C*, even in the experiments in which *D* closed eight hours before *C*. As was the case in the experiments made by Morgan, an interval of eight hours between the two cuttings has, apparently, no more effect on the regeneration than has an interval of only two hours. The formation of a polyp at the oral end, *C*, of the proximal piece, *CD*, does not prevent the early development of a polyp at the aboral end of the same piece, and in some cases there is only a few hours interval between the formation of the two hydranths. The earlier development of a hydranth at *A*, however, seems to check the formation of a hydranth at the aboral end, *B*, of the distal piece for some time, as in no case did a hydranth develop at *B* until five days after the experiment began. This difference in the rate of development at *D* and at *B* cannot be due to a difference in the lengths of the pieces, because, in making the experiments, the stems in all cases were cut as nearly as possible through the middle and any difference in the lengths of the anterior and of the proximal pieces would be too slight to have any appreciable influence on the result. The earlier closing of the aboral end, *D*, of the proximal piece, *CD*, evidently counterbalances to some extent the influence of the oral end, as suggested by Morgan. As a result, the development of a polyp at *D* is hastened somewhat, although in no case is a hydranth formed here before or as soon as one develops at the oral end of the piece.

The effect of the earlier closing of the aboral end of long pieces of the stem, in both *Tubularia mesembryanthemum* and in *Tubularia crocea*, is to hasten the development of the aboral surface. The influences that bring about this result are apparently not as strong in the latter species as in *Tubularia mesembryanthemum* where the aboral development may be hastened so much that polyps develop simultaneously at both ends of the piece.

This seeming difference between the two species may possibly be due to the fact that *Tubularia crocea*, which lives in cold water, regenerates very slowly and, therefore, comparatively slight differences in the rate of regeneration at the oral and aboral ends of the stem can be readily noted. *Tubularia mesembryanthemum*, on the other hand, lives in much warmer water and its regeneration takes place so quickly that it is difficult to detect slight differences in the rate of development of the polyps at the cut oral and aboral surfaces.

In a variation of the above experiment, a piece of silk thread was tied tightly around the stem about 2 mm. below the hydranth, and another piece was tied about 30–40 mm. below the first. Both ends of a long piece of stem were, therefore, closed at practically the same time in such a way that no regeneration was possible from either end of the piece. After the ends had been tied, the stem was cut transversely through the middle as in Fig. 1, *B*, *C*, in order to ascertain whether subsequent regeneration from the cut surfaces, *B*, and *C*, would be hastened in comparison with the rate of regeneration from similar surfaces of pieces of stem of the same length, cut at the same time, but not closed artificially at one end. The control pieces of stem were kept in the same dishes with those used in the experiment, and both sets, therefore, were under the same external conditions.

Eight long pieces of stem were used in this experiment. Two days after the operation, tentacle anlagen had appeared at the cut ends of all of the sixteen pieces, but they were not as well developed on the aboral end, *B*, of the anterior piece as they were on the oral surface, *C*, of the posterior piece. At this time there was no indication of any regeneration at the aboral surface of the anterior piece in the control set of stems, although in some cases complete hydranths, in other tentacle anlagen, were present on the oral end of the proximal pieces. On the third day after the operation, polyps were found on the oral end, *C*, of all of the proximal pieces, both in the control and in the tied stems. The development from the aboral surface, *B*, of the anterior pieces, however, did not keep pace with that at the oral end, *C*, of the proximal pieces, as at this time only two of the pieces of stem tied at one end had produced hydranths at the aboral sur-

face, *B*, the rest had, as yet, developed only tentacle anlagen ; in the control stems, no development from the aboral surface, *B*, had taken place in any case.

It is seen from the above experiments, that the development of a hydranth at the oral end of a piece of the stem of *Tubularia crocea* is not hastened by artificially closing the aboral end. Tying the oral end of a distal piece of the stem, however, hastens the development of the aboral end of the piece as compared with the development that takes place from the aboral surface of a piece of stem of similar length that is not closed at the oral end, as Driesch has shown. This result also agrees with that obtained by Loeb (7) in experiments in which he stuck the oral end of pieces of the stem of *Tubularia mesembryanthemum* in fine sand, leaving the other end freely surrounded by water. He found that "Durch Hemmung der Polypenbildung am oralen Ende kann man also die Polypenbildung am aboralen Ende beschleunigen."

In a third set of ten experiments, hydranth bearing stems about 30 mm. in length were removed from the colony and kept until a polyp formed on the cut aboral end. The time required for the development of these aboral polyps varied from three to five days in different cases. After all of the pieces had developed hydranths at the aboral end, each stem was cut transversely through the middle as in Fig. 2, *B*, *C*. The object of these experiments was to ascertain whether the presence of a hydranth at the aboral end, *D*, of the proximal piece, *CD*, would alter the polarity of the piece and thus prevent or retard the development of a hydranth at the oral end, *C*. The stems were cut through the middle region on June 19. Not until June 22 were there any indications of a development of a hydranth at the oral surface, *C*, and then only faint traces of tentacle anlagen were found in three stems. At this time, no development had taken place from the aboral surface, *B*, of any of the anterior pieces, *AB*. For control experiments, pieces of stem about 30 mm. in length were cut through the middle as in Fig. 1, *BC*, at the same time that the transverse cuts were made across the stems bearing a hydranth at each end, and on June 22, well developed hydranths were present at the oral end of all of the proximal pieces. On

June 23, three of the ten proximal pieces of stem bearing aboral hydranths had also developed hydranths at the oral end; while the oral end of two other pieces of stem showed well developed tentacle anlagen which developed into hydranths on the following day. No further changes took place in any of the pieces although they were kept for some ten days longer.

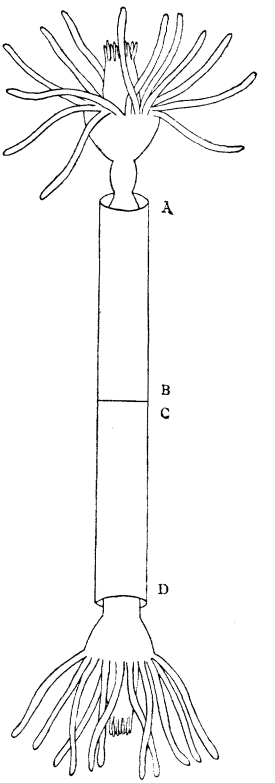

FIG. 2.

It is seen from the above experiments, that the presence of a hydranth at the aboral end of a piece of the stem of *Tubularia* delays, but does not prevent, the development of a hydranth at the oral end of the piece. This result cannot be due simply to the fact that the proximal end of the piece was closed by the presence of the aboral polyp, because, in the previous set of experiments, it was shown that closing the aboral end of a piece of stem by tying does not delay the development of a polyp at the oral end. It seems probable that the polarity of the piece was changed, for a time at least, by the presence of a hydranth at its aboral end and, therefore, the influences for hydranth formation at the freshly cut oral surface were not strong enough to bring about the development of a polyp for some days.

II. EXPERIMENTS ON BRANCHING STEMS.

The following series of experiments were made in order to ascertain whether the development of a hydranth on the oral end of a stem will influence the rate of development of a hydranth on the distal end of a long or of a short piece of a branch, and also to determine what conditions are necessary in order that the formation of a hydranth at the one place will prevent the formation of a hydranth at the other.

Series 1. — On June 24, twenty experiments were made in which a branch was cut off about 1 mm. from its origin in the stem, and then the anterior end of each stem was removed by a transverse cut leaving a piece from 10–20 mm. above the place of union with the branch (Fig. 3). In one case, a hydranth developed on the cut end of the branch two days after the operation, and at the same time a polyp also formed on the oral end of the main stem which in this instance was 20 mm. in length above the origin of the branch. In all other individuals at this time tentacle anlagen had formed at the oral end of the stem, but there was no indication of the development of a hydranth at the cut end of any of the branches. On June 27 hydranths were found at the oral end of all of the stems and also on the distal end of four branches; all the remaining branches had well developed tentacle anlagen excepting one which showed no signs of regeneration during the course of the week that the hydroids were kept. In this set of experiments, therefore, with the exception of the one case noted, regeneration of a hydranth took place at the distal end of the long stem before a polyp formed at the oral end of the short branch.

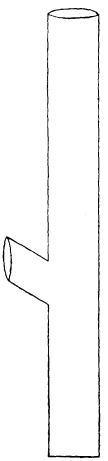

FIG. 3.

The results of this set of experiments might possibly be considered to be due to the fact that the longer piece exerted some kind of an influence over the shorter piece that would tend to alter the polarity of the shorter piece and thus retard development from its cut oral surface. That a larger piece of a hydrozoa can influence the polarity of a smaller piece is shown unquestionable in grafting experiments that I made on *Hydra viridis* (King, 6) in which the larger component of the graft either absorbed the smaller component or formed a permanent union with it. In the latter case, the polarity of the smaller piece was completely reversed, if necessary, in order that a structure might regenerate on its cut surface that would produce a normal polyp. Another factor that might, possibly, cause a delay in the development of a hydranth from the cut surface of the shorter piece is the length of the piece. Morgan has shown that in

branching stems a short piece of a branch or of a stem regenerates as does an isolated short piece, *i. e.*, the region of the tentacle anlagen is reduced and the rate of development of a hydranth is much slower than that of a hydranth from the cut end of a long piece of stem. Both of these factors may help to bring about the delay in the development of a hydranth from the oral end of the shorter piece in all of these experiments with branching stems in which there is a marked difference in the length of the stem and of the branch.

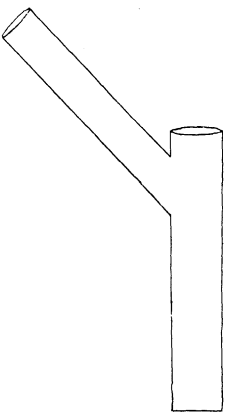

FIG. 4.

Series 2. — Seventeen branching stems from different colonies were cut so that the anterior portion of the stem above the place of union with the branch was only about 1 mm. in length, while the length of the branch varied in different cases from 8–25 mm. (Fig. 4). Two days after the operation, hydranths had developed at the cut ends of thirteen branches, and well developed tentacle anlagen were found at the oral ends of the other four branches. No hydranths were found at this time at the oral end of any of the stems, and tentacle anlagen were only faintly defined in some few cases. On the next day, hydranths had developed at the distal end of all of the branches, but only five stems bore hydranths at the oral end. In this set of experiments the development of a hydranth took place more rapidly from the cut end of the long branch than from the oral end of the main stem.

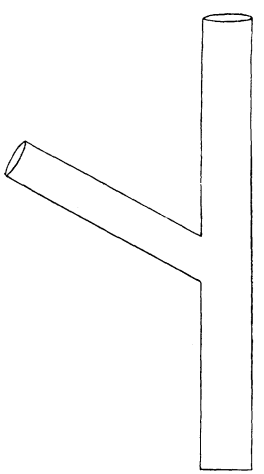

FIG. 5.

Series 3. — Twelve experiments were made in which the anterior end of the stem and the distal end of the branch were cut off so that the length of the branch and of the stem above the place of union was practically the same, varying in different cases

from 8–20 mm. (Fig. 5). Two days after the operation, there were well developed hydranths on the ends of three branches and only tentacle anlagen on the oral ends of the corresponding stems. In two cases hydranths had developed on the end of the branch and also on the cut surface of the stem; while in the other cases only tentacle analgen were found, and they were equally well-developed on the branch and on the oral end of the stem. During the following two days, hydranths formed on all of the cut ends, sometimes the hydranth developed on the end of the stem before it did on the branch, and sometimes the hydranth appeared first on the branch. As a general result of this series of experiments it can be stated that hydranths develop at about the same rate when both the branch and the anterior portion of the stem are approximately the same length.

Series 4. — In the previous set of experiments both the branch and the anterior portion of the stem were of considerable length and both developed at about the same rate. In order to see if similar results would be obtained if the pieces were very short, fifteen experiments were made in which the branch and the stem were cut off about 1 mm. above their point of union (Fig. 6). When the hydroids were examined three days after the operation, hydranths were found on the oral end of the stem and not on the cut end of the branch in five cases; while in four hydroids, polyps had developed on the branch and not on the oral end of the stem; in the remaining six cases, hydranths were present at the distal end of both branch and stem.

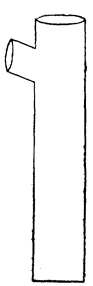

FIG. 6.

In those cases in which one or the other cut surface failed to develop a hydranth, the cœnosarc appeared to be entirely with drawn from this part. When these stems were examined under the microscope, the streaming of granules in the interior cavity was visible only in the proximal part of the main stem and in the part of the stem or branch that had regenerated. In the cases in which hydranths formed at both cut surfaces, the streaming of granules was found in all parts of the stem and also in the branch.

In this set of experiments, regeneration seemed to take place

with equal rapidity from the cut oral surface of the branch and of the stem, as was the case in the experiments described under series 3. If the pieces above the place of union of the branch and stem are of approximately the same length, no matter how long or how short they may be, the influences for hydranth formation appear to be alike in both, and one piece has, seemingly, no effect on the other. Where the cœnosarc is withdrawn entirely from one part of the hydroid, as was noted in some few cases in the last set of experiments, no regeneration of the piece is possible.

In experimenting on *Tubularia crocea*, Morgan (9) found that if he cut off both the main stem and the branch a little above the place of union, the results varied considerably in different cases. In some instances he obtained the regeneration of a hydranth on the branch and not on the oral end of the stem; in other cases a polyp formed only at the oral end of the main stem; and in still other individuals regeneration took place from the cut surfaces of both branch and stem. It seems probable that the lack of uniformity in these results can be attributed to a difference in the relative lengths of the branch and of anterior portion of the stem above the point of insertion of the branch. It is, of course, impossible to cut the branch and stem at absolutely equal distances from the place of their union, and in those cases in which regeneration from one cut surface took place before it did from the other, there may have been just enough difference in the lengths of the pieces to bring about the earlier regeneration of a hydranth on the cut oral surface of the longer piece.

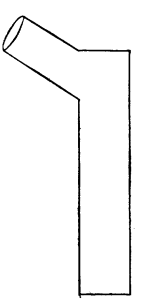

FIG. 7.

Series 5. — In twenty-eight cases the stem was cut off transversely just above the origin of the branch as shown in Fig. 7. The oral end of the branch was then removed leaving a piece, from 3 to 5 mm. in length, still attached to the stem. The object of these experiments was to see whether the regeneration of a hydranth at the oral end of the stem could be entirely prevented by this means. Two days after the operation, hydranths had developed at the cut end of the branch in nine of the hy-

droids and well-developed tentacle anlagen were present on the other branches; there was no indication of a regeneration at the oral end of any of the stems. The next day all of the branches bore hydranths, and in but one case had any regeneration taken place at the oral end of the stem. In this instance, the stem extended about 0. 5 mm. above the place of insertion of the branch and a considerable amount of red pigment had collected at its extreme oral end. In the course of forty-eight hours more a polyp formed on the oral end of this stem but no regeneration took place at the oral end of any of the other stems, although they were kept for over a week.

Series 6.— Sixteen experiments were made in which the branches were cut off very close to their origin on the stem. The oral end of the stem was then removed leaving a piece about 5 mm. in length above the origin of the branch, in order to see whether the formation of a hydranth at the oral end of the stem would prevent or merely delay the formation of a polyp at the place where the branch was removed. In all cases the wound in the side of the stem healed over very quickly and, although the hydroids were kept alive for a number of days, no regeneration of any kind took place at the point of injury.

Series 7. — In ten cases the entire branch was removed from the stem, but the old hydranth at the distal end of the stem was not cut off. The result was the same as in the previous set of experiments, as the cut surface was very soon covered over and no subsequent regeneration took place from it.

Series 8. — In sixteen cases where long pieces of stem bore from two to four branches, the anterior end of the stem and the apical end of each branch were removed by transverse cuts leaving the lengths of the branches approximately the same as that of the stem above the origin of the most anterior branch. The experiments were made to see if there is any difference in the relative rate of regeneration of the anterior branches and of the proximal ones. There was no uniformity whatever in the results of this set of experiments. In some cases a hydranth regenerated on a posterior branch before it did on the oral end of the main stem; and in other cases all of the branches produced hydranths at the same time that one developed at the oral end of the stem.

III. The Regeneration of Short Pieces of the Stem of Tubularia.

It was first noted by Bickford (1), and later confirmed by Driesch (2) and by Morgan, that small pieces of the stem of *Tubularia* about 1 mm. in length are capable of regenerating. In a recent paper, Hargitt (5) states that he was unable to obtain any regeneration from pieces of the stem of *Tubularia crocea* and of *Tubularia tenella* that were as much as 3–4 mm. in length. This result was probably due to the poor condition of the stems when the experiments were made. Small pieces of the stem of some other hydroids, do not appear to possess as great a power of regeneration as *Tubularia*, for Gast and Godlewski (4) have found that pieces of the stem of *Pennaria cavolinii* about 1 mm. in length never produce hydranths and pieces 2 mm. in length regenerate hydranths but rarely. Bickford's experiments on small pieces of the stem of *Tubularia tenella* show that, in this species, regenerative processes are not restricted to any special region of the stem, and also that such short pieces tend to form one complete hydranth rather than to produce double abnormal structures.

In experimenting on *Tubularia mesembryanthemum*, Driesch (2) found that of 82 short pieces of stem, 5 formed a single proboscis, 26 formed a double proboscis, and the remaining 51 pieces produced hydranths. These results agree with those of the earlier experiments of Bickford. In a later paper, Driesch (3) states that at the oral end of the stem one seldom gets a whole hydranth, but usually a single or a double proboscis; from the middle zone hydranths usually develop; while from the aboral end of the stem, these structures are rarely produced. This difference in the kind of regeneration from the various parts of the stem Driesch attributes to the situation of the small piece in the original individual and to the different distribution of the hydranth-forming pigment in the cœnosarc of the different parts. Since Morgan and also Stevens (11–12) have proven the fallacy of the hypothesis of "red formative stuff" in *Tubularia*, this portion of Driesch's explanation is, of course, no longer tenable.

Morgan (8–10) has made an extended series of experiments with small pieces of the stem of both *Tubularia mesembryanthemum*

and of *Tubularia crocea.* He finds, as did Driesch, that pieces about 1 mm. in length from the region immediately behind the old hydranth usually die, even when longer than pieces from a more proximal region that regenerate. When this distal region does regenerate, it produces a greater number of single proboscides than of other forms, a result that might be expected as this part of the stem ordinarily goes into the proboscis of the new hydranth when a long piece of stem is regenerating.

In another set of experiments, Morgan cut pieces of the stem of *Tubularia mesembryanthemum* into a series of small pieces about 1 mm. in length in order to observe the behavior of consecutive pieces from one stem and to compare the results with those obtained from similar pieces cut from other stems. His tables do not show any very definite results although there seems to be a certain similarity in the behavior of pieces of the same stem, and the incomplete structures are found most frequently at the distal end of the stem.

At the suggestion of Professor Morgan, I repeated his experiments, using *Tubularia crocea,* in order to furnish more data from which definite conclusions could be drawn. In making the experiments the old hydranths were removed and the distal part of the stem was cut into consecutive pieces about 1 mm. in length. The pieces were than laid in rows on the bottom of flat dishes filled with fresh sea water. For the sake of brevity the following abbreviations are used in the tables given: hy. = complete hydranth without any stalk; hy. + stalk = hydranth with a short stalk that has been formed by a withdrawal of the cœnosarc from the perisarc; hy. + stem = hydranth with a stem attached to the perisarc; pb. = single proboscis; d. pb = double proboscis; reprod. = reproductive organs. The results tabulated are from observations made three to four days after the operation.

TABLE V.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb. with no tentacles.	6	pb.
2	d. pb.	7	d. pb.
3	pb.	8	hy. + stem.
4	d. pb. + reprod.	9	d. pb.
5	pb.	10	d. pb.

TABLE VI.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	dead.
2	d. pb.	7	pb.
3	d. pb.	8	pb.
4	dead.	9	dead.
5	d. pb.	10	dead.

TABLE VII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	d. pb.	6	d. pb.
2	pb.	7	d. pb. + reprod.
3	pb.	8	pb.
4	pb.	9	hy. + stem.
5	hy.	10	pb.

TABLE VIII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kird of Structure Regenerated.
1	dead.	6	pb.
2	d. pb.	7	pb.
3	d. pb.	8	hy. + stalk.
4	pb.	9	pb.
5	pb. + reprod.	10	d. pb.

TABLE IX.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb. + reprod.
2	pb.	7	pb.
3	pb.	8	hy. + stem.
4	hy. + stem.	9	pb.
5	pb.	10	dead.

TABLE X.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	pb.
2	d. pb.	7	hy. + stem.
3	d. pb.	8	pb.
4	d. pb. + reprod.	9	hy.
5	pb	10	dead.

As was the case in the experiments made by Morgan, the results for corresponding pieces of different stems are far from uniform, and it is not possible to determine what kind of a structure will be produced by a small piece from a given region of the stem. It is evident that the power to form either complete or double structures is present throughout the stem, and just what conditions are necessary to produce certain structures have not, as yet, been fully determined. Morgan has suggested that possibly the factors in determining the kind of regeneration are (1) the smallness of the piece, (2) the differences in the region of the original stem from which the pieces came (this factor had been previously suggested by Driesch), (3) the age of the piece, as the younger the stem the more likely it would be to form incomplete structures.

According to this set of experiments short pieces, no matter from what part of the stem they are taken, are more liable to produce proboscides than to form hydranths. When the latter structures appear they are usually produced by the more proximal pieces of the stem, the distal end of the stem showing a great tendency to produce incomplete structures. These results are very similar to those obtained by Morgan on *Tubularia mesembryanthemum.*

In order to ascertain whether the double structures that are so often obtained in such experiments are produced because the small pieces of the stem are open at both ends and not because there is insufficient material in the piece to produce a complete hydranth, Morgan tied one end of a short piece with silk thread, and found that, under these conditions, double structures are never produced. Later he planted short pieces of stems in rows in sand so that one end was buried and the other freely surrounded by water. In two instances only was a double proboscis formed, in all other cases single structures, either incomplete or whole, more often the latter were produced.

In repeating these experiments of closing one end of a short piece of the stem of *Tubularia* in order to ascertain the effect on the kind of structure produced, the following method was used: Shallow, flat dishes were covered on the bottom with a layer of paraffine about one fourth of an inch in thickness, and then, with

the blunt end of a large needle about the diameter of a tubularian stem, rows of holes were made in the paraffine about 0.5 mm. in depth. The dishes were filled with water and the long pieces of stems were put in them and cut into consecutive pieces about 1 mm. in length. One end of each piece was then inserted in a hole that was just large enough to receive and hold it upright. This method has the advantage that the inserted end of the piece of stem is in contact with the paraffine and cannot become free if the experiment is properly done. The results of this series of experiments are summarized in the following tables in which the abbreviations used are the same as those previously employed.

TABLE XI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb.	6	pb.
3	pb. + reprod.	7	dead.
4	dead.	8	pb.

TABLE XII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy.	7	pb.
4	dead.	8	dead.

TABLE XIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	dead.	6	pb. + reprod.
3	d. pb.	7	pb.
4	dead.	8	pb.

TABLE XIV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	hy.	5	dead.
2	pb.	6	dead.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	dead.
3	pb. + reprod.	7	dead.
4	pb.	8	dead.

TABLE XVI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XVII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb. + reprod.	6	dead.
3	pb.	7	dead.
4	pb. + reprod.	8	dead.

TABLE XVIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	dead.	5	dead.
2	pb.	6	hy. + stalk.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XIX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	dead.
3	hy. + stalk.	7	hy. + stem.
4	pb.	8	hy. + stem.

TABLE XX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy. + stem.	7	pb.
4	dead.	8	dead.

TABLE XXI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XXII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	dead.	6	hy. + stalk.
3	pb. + reprod.	7	pb.
4	pb.	8	dead.

The results of these experiments confirm those obtained by Morgan in every respect, as double structures were produced very rarely, only one being obtained in the entire series of experiments. There seemed to be no distinctive individual differences in the pieces of stem as regards the structures produced. In only one case (Table XIX.) were as many as three hydranths produced, while in the tables given by Morgan for *Tubularia mesembryanthemum*, whole series of pieces from the same stem produced hydranths.

If a comparison is made between the results shown in Tables XI. to XXII. and those shown in Tables V. to X., the most noticeable difference is that a very much greater number of double structures were produced when short pieces of the stem were lying on their sides during the process of regeneration. In both sets of experiments single proboscides were the structures most frequently produced, and very little individual difference could be detected in the stems regarding the kind of structure that they would tend to produce.

The development of small pieces of the stem of *Tubularia* standing on one end is considerably slower than that of similar pieces lying on one side. In the latter case, development takes place in about two days; while in the former case the various structures never appear under three days, and usually not under four or five days. Many pieces, usually those nearer the proxi-

mal end of the stem, die when the pieces stand on one end. This result may be due to the fact that the conditions under which regeneration takes place are not as favorable when the end of a small piece of stem is closed by contact with some foreign substance, as when the piece lies on one side and the cut ends are allowed to close in a normal manner.

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Bryn Mawr, Pa., March 23, 1904.

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